EXPRESS MAIL CERTIFICATE

Express Mail Certificate No. EV268203996US
I hereby certify that the *attached* correspondence comprising:
1). Declaration under 37 CFR §1.131 (3 pages) w/attachments (12 pages) 2). Return postcard is being deposited with the United States Postal Service as "Express Mail Post Office to addressee" under 37 CFR 1.10 on the date indicated below in an envelope addressed to:
Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

July 18, 2003

Kathy Raymond

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant :	Robin R. Miles, et al.	Docket No. :	IL-10632
Serial No. :	09/733,857	Art Unit :	1753
Filed :	December 8, 2000	Examiner :	Alexander Stephan Noguerola
For :	DIELECTROPHORETIC CONCENTRATION OF PARTICLES UNDER ELECTROKINETIC FLOW		

DECLARATION UNDER 37 CFR §1.131
Declaration by Robin R. Miles, Kerry A. Bettencourt, and Christopher K. Fuller to Overcome Cited Reference

Commissioner of Patents and Trademarks
Alexandria, VA 22313-1450

Dear Sir:

We; hereby declare that:

(1) We, Robin R. Miles, 80 Kendall Lane, Danville, Ca 94526; Kerry A. Bettencourt, 11873 West Vomac Road, Dublin, CA 94568; and Christopher K. Fuller, 4224 Atlas Avenue, Oakland, CA 94619; are citizens of the United States and residents of the Cities and the State identified;

(2) We are the inventors named in the subject application;

(3) Claims in the subject application were rejected over the McBride et al Reference, United States Patent No. 6,296,752 issued October 2, 2001 from an

application filed June 4, 1999, therefore, June 4, 1999 is the filing date of the application from which the McBride et al Reference matured;

(4) We made the invention described and claimed in the subject patent application (The Invention) in this country prior to June 4, 1999, having made drawings, having made written descriptions, having made tests, and having disclosed the invention to others; all of the foregoing having been done in this country prior to June 4, 1999; and We continuously worked on testing, developing, and patenting The Invention during the period from the time when We made the first written description of The Invention and disclosed The Invention to others until December 8, 2000 when the subject application was filed (hereinafter "The Time Period");

(5) We conceived and reduced The Invention to practice in this country prior to June 4, 1999; attached as ATTACHMENT A is photostatic copy of a "RECORD OF INVENTION" which in the Conception of the Invention Section XI, contains an entry for the "Conception Date" and an entry for "First Written Description," and in the Reduction to Practice of the Invention Section XII, contains an entry for the "Date of Operation and Testing;" the dates on the photostatic copy of ATTACHMENT A have been blacked out, however, dates showing that We made The Invention prior to June 4, 1999 are dates prior to June 4, 1999; that We conceived The Invention in this country are dates prior to June 4, 1999; that We reduced The Invention to practice in this country are dates prior to June 4, 1999, and dates showing that The Invention was continuously worked on during The Time Period are dates during The Time Period;

(6) We made drawings, made written descriptions, and made tests, of The Invention in this country prior to June 4, 1999; attached as ATTACHMENT B is photostatic copy of "pages from Robin Miles' Laboratory Notebook" showing

We made drawings, made written descriptions, and made tests of The Invention in this country prior to June 4, 1999;

(7) We made drawings, made written descriptions, and made tests, of The Invention in this country prior to June 4, 1999; attached as ATTACHMENT C is photostatic copy of "pages from Kerry Bettencourt's Laboratory Notebook" showing We made drawings, made written descriptions, and made tests of The Invention in this country prior to June 4, 1999;

(8) We do not know and do not believe that the invention has been in public use or on sale in this country, or patented or described in a printed publication in this or any foreign country for more than one year prior to our application, and we have never abandoned our invention;

(9) We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

7/17/03

(Signature) July __, 2003
Declarant: Robin R. Miles

7/10/03

(Signature) July __, 2003
Declarant: Kerry A. Bettencourt

7/6/03

(Signature) July 6, 2003
Declarant: Christopher K. Fuller

ATTACHMENT A

University of California
Lawrence Livermore
National Laboratory

RECORD OF INVENTION

LLNL File No.

IX. Inventor Information

Inventor's Permanent Home Address (Please attach a separate sheet for additional inventors.)

Full Name	Citizenship	Street Address	City, State, Zip Code
Robin R. Miles	USA	826 Cherokee Drive	Livermore,CA 94550
Kerry Bettencourt	USA	11873 West Vomac Road	Dubin, CA. 94568
Christopher Fuller	USA	1134 Portola Meadows Apt. 216	Livermore, CA 94550

X. Funding Source

Funding Source or Project Under Which the Invention Arose (Include subcontracts, CRADAs, international agreements, work for others, or special project information.):

Resource Manager	Phone #	Is funding presently being provided for development of your invention?	Yes	No
Pam Richmond	2-4965	[redacted]	X	

LLNL Acct #	B&R #	Please state the source of funds (if same as above, please so state):		
8989-76	[illegible]			
Subcontract #	DOE Program Code	Do you reasonably expect future funding from the current source or other sources?	Yes	No
		[redacted]		
CRADA #	Work for Others #	If yes, what is that source?		

XI. Conception of the Invention

Conception Date	Conception Place		
	Livermore CA.		
Earliest documentation of your invention (please provide date and identify the document): LDRD proposal		First Sketch or Drawing Date	First Written Description Date 2/98

Names of Witnesses or others with knowledge of facts relating to conception (preferably at least 2):

Full Name	Organization	Telephone Number
Ray Mariella	LLNL	2-8905
Harold Ackler	LLNL	2-6235

XII. Reduction To Practice of the Invention

Date first model completed	Date of operation and testing	Place of test
		LLNL, Livermore CA

Results of testing:
Worked.

Witnesses or others with direct knowledge of test (preferably at least 2):

Full Name	Organization	Telephone Number
Kerry Bettencourt	LLNL	2-7371
Robin Miles	LLNL	2-5048
Chris Fuller	LLNL	4-5185

University of California
Lawrence Livermore
National Laboratory

RECORD OF INVENTION

LLNL File No.

XIII. Invention Use and Disclosure

Has the invention been put into use?	Yes	No	If yes, explain:
		X	

Has the invention been disclosed to non-LLNL personnel?	Yes	No	If yes, to whom and when? Provide name(s) and date(s):
		X	

If yes, was the disclosure done under a non-disclosure agreement?	Yes	No

Planning to write paper Jan '00

XIV. I/We believe myself/ourselves to be the first and original inventor(s) of the above-described invention.

Inventor Signature	Date	Witness Signature	Date
		Cheryl Stockton	
		S Balasubramanian	

XV. Classification Review

Basis for unclassified release:		
X	Outside scope of AEA and EO	
	CG-DAR-1, Topic(s):	
	Other Guide(s):	
Topic(s):		

UCNI	Yes	No	If YES, Guide:
[redacted]			

Authorized Derivative Classifier -- Name and Title	Signature
M. D. POCHA SECTION LEADER	M. D. Pocha
Confirming Reviewer -- Name	Signature

XVI. For LLNL Patent Group Use Only

Possible Statutory Bars

Publication	
Public Use/Sale	
Recommended Filing Date Due to Possible Statutory Bars	

Preliminary Review By:	Date

Send the completed and signed form to the Patent Group at L-703

University of California
Lawrence Livermore
National Laboratory

RECORD OF INVENTION

LLNL File No.
IL 10632

XIII. Invention Use and Disclosure

Has the invention been put into use?	Yes	No	If yes, explain:
		X	

Has the invention been disclosed to non-LLNL personnel?	Yes	No	If yes, to whom and when? Provide name(s) and date(s):
		X	-

If yes, was the disclosure done under a non-disclosure agreement?	Yes	No

Planning to write paper

XIV. I/We believe myself/ourselves to be the first and original inventor(s) of the above-described invention.

Inventor Signature	Date	Witness Signature	Date
[signature]		Cheryl Stockton	
[signature]		[signature]	
[signature]		S L Vasconcellos	

XV. Classification Review

Basis for unclassified release:		
X	Outside scope of AEA and EO	
	CG-DAR-1, Topic(s):	
	Other Guide(s):	
Topic(s):		

UCNI	Yes	No	If YES, Guide:
[redacted]			

Authorized Derivative Classifier -- Name and Title	Signature
M. D. POCHA SECTION LEADER	M. D. Pocha
Confirming Reviewer -- Name	Signature
Wm. A. BOLLINGER	Wm. A. Bollinger

XVI. For LLNL Patent Group Use Only

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Publication	
Public Use/Sale	
Recommended Filing Date Due to Possible Statutory Bars	

Preliminary Review By:	Date

Send the completed and signed form to the Patent Group at L-703

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Lawrence Livermore
National Laboratory

RECEIVED

RECORD OF INVENTION

LLNL – I. P. L. G.

LLNL File No.
IL- 10632

This invention was made in the course of or under prime Contract No. W-7405-ENG-48 between the U.S. Department of Energy and the University of California. This Record of Invention is prepared for the Office of the Assistant General Counsel for Patents, U.S. Department of Energy.

I. Title of the Invention

Dielectrophoretic Concentration of Particles under Electrokinetic Flow

II. Inventor Information

LLNL Inventor(s) (F M L)	Title/Position	Directorate	Payroll Acct	Phone #	Mail Stop
Robin Miles	Engineer	Engineering	9782	2-5048	L-223
Kerry Bettencourt	Chemistry Associate	Chemistry	9811	2-7371	L-223
Chris Fuller	Engineer	Engineering	9782	4-5185	L-223

III. Abstract

The use of dielectrophoresis to collect particles is well known when operating under pressure driven flow. However, to our knowledge, no one else has documented such collection under the conditions of electrokinetically-driven flow. Electrokinetically-driven flow is an important technique for moving fluids and sample around a microfluidic bio-chemical assay chip. We have now shown that it is possible to reap the advantages of dielectrophoretic manipulation in this regime.

IV. Uses of the Invention

List past uses, current uses and potential uses for your invention

LLNL or Government uses or possibilities for use:

Dielectrophoresis is used to effect motion on polarizable particles within a non-uniform electric field. Positive dielectrophoresis can be used to concentrate particles in areas of high electric field gradients. This can be used to eliminate the use of centrifuging to concentrate biological samples. Negative dielectrophoresis can be use to discriminate between various types of biological particles.

Commercial or other uses or possibilities for use:

Companies using microfluidic devices to perform analysis would be interested in using this device to concentrate the sample prior to testing.

LLNL File No.

V. Documents Describing th Invention

Documents, publications, and presentations describing the invention that you have published or prepared for publication, or presented on the subject. Also include presentations and publications planned within one year from now. Please attach a copy of preprints, articles, or viewgraphs.

Title/Subject	Date	Publication #
None.		

VI. Documents Describing Prior Art (Please include copies of these documents.)

R lated Documents (including patents, other publications) Please include patent numbers, authors, title, publication date, etc.

Many publications discuss dielectrophoretic concentration, but none that we know about use electrokinetic/electroosmotic-driven flow.

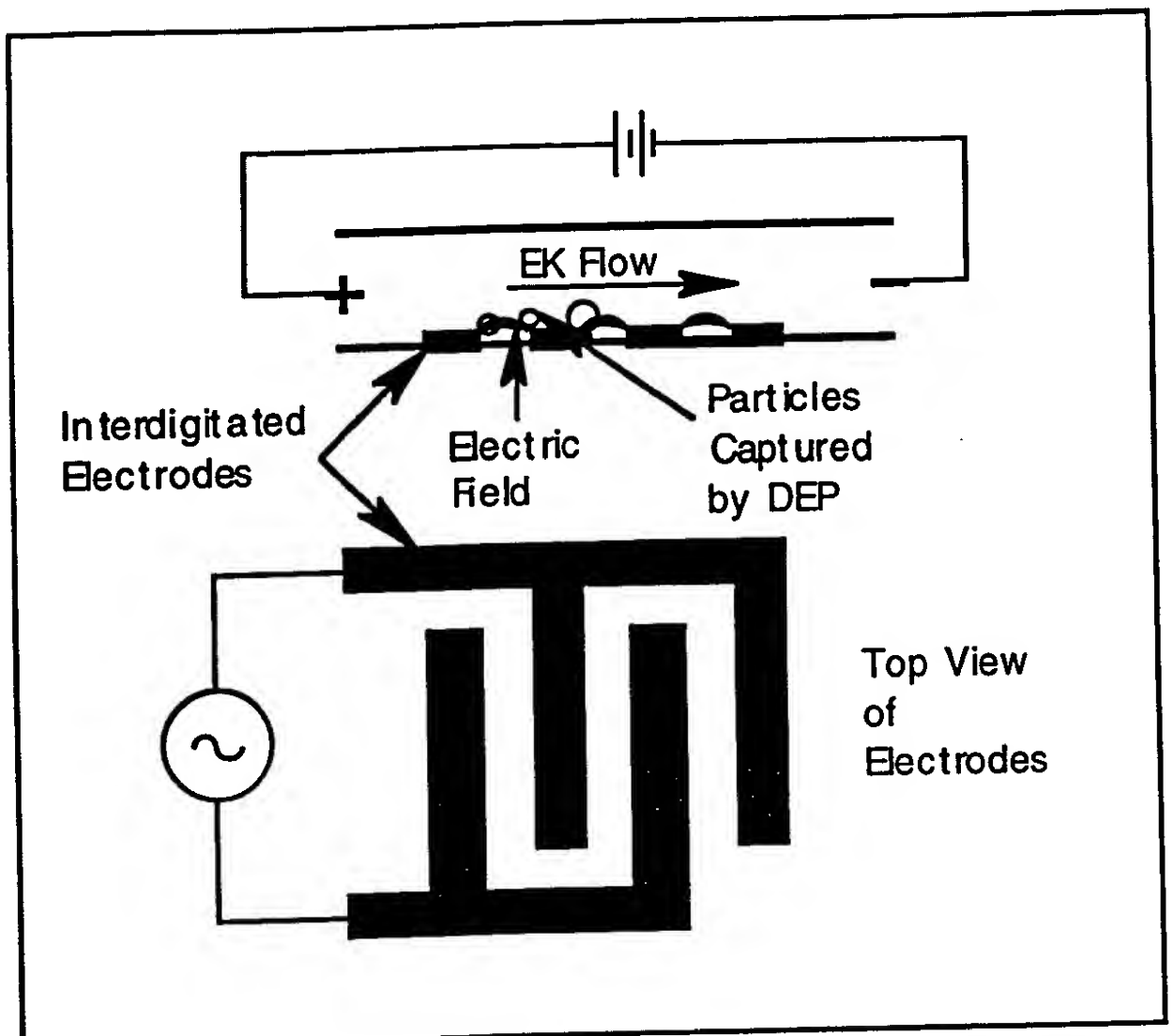
VII. Background

Background of the invention, including technical problems addressed by it:

Microfluidic devices are most useful when operating with small sample volumes. Small sample volumes result in increased reaction times and reduced reagent use which means significantly reduced costs for the multitude of tests that one desires to conduct on any given sample. Dielectrophoretic concentration of the sample is a useful method for achieving these goals. Dielectrophoresis is attractive on the microfluidic scale because the forces become significant and useful at dimensions of less than a 1 mm.
Electrokinetic/electroosmotic flow is also useful in these devices because it obviates the need for micropumps and microvalves. The combination of dielectrophoresis and electrokinetic/electroosmotic flow would not normally be an obvious choice since one might think that the two electric fields and their associated double layers may interfere with each other. Also, DEP collection works best in the slow boundary-layer flow associated with pressure-driven flow. We have found that particles can still collect even in the more uniform flow field associated with electroosmotic flow. The 5-10 nm double charge layer associated with establishing electroosmotic flow does not seem to interfere or be interfered with by the DEP field in a slgnificant way.

VIII. Invention Description

Description of the invention (you may also attach a paper). Please include a sketch of the invention, if possible.



A schematic of the device is shown above. Interdigitated electrodes are patterned on the inner surface of a microfluidic channel. Glass is the preferred material for the microfluidic channel because it promotes electroosmotic flow, particularly if preconditioned with sodium hydroxide. A DC voltage is applied across the ends of the channel to initiate the electrokinetic/electroosmotic flow field. An AC voltage is applied across the interdigitated electrodes to set up a non-uniform electric field capable of trapping particles using the dielectrophoretic force. Particles are swept down the channel electrokinetically and are trapped within the field established by the interdigitated electrodes. The particles can be released when the voltage to the interdigitated electrodes is released.

EO flow + electrode

400 V across ~ 10 cm,
3.8 V, 1 Khz 1 + 5 μm beads :- PH 8.0 solute
pump w/ NaOH soln.

pattern faded w/ Nitric

fair amount of bubbles → EO stopped bubbles on
electrodes is out.

Step ① ⓐ Nothing
turn on EO/ turn on DEP.
Collect zero bubbles? too? (a) tag needs

②
pattern.
DEP increase field. - boiling
change electrode set

collect @ 3.8 V → good collection
start EO field. , 500 V
stuff still on edge after flow
turn off one side → collect at other side
release & re collect

EO Flow then DEP → stuff on electrode but not
near edge. → release.

low stepper

300 V → set voltage, put on field
3.8 V, 1 kHz → demonstrated collect & release
w/ EO flow.

bubble at entrance stop flow →

a few small generated bubble not redistributed electrodes

→ Run 2, 100 V 3.5 V 1 KHz

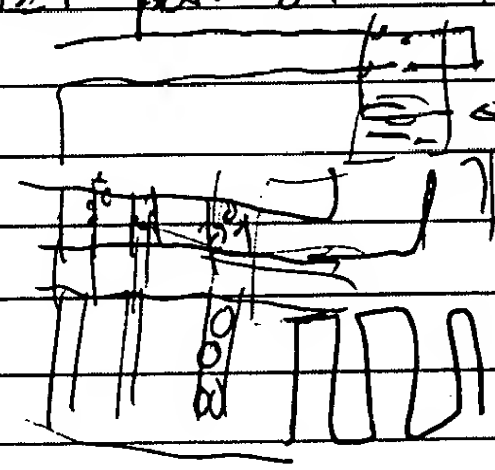
pH 8.0, 10% between .05% = .05%

the 200 V 3.4 → 3.5 V

massive bubbles. (then 400 V 3.5 V
boiling bubbles.

< bubble 500 V → 3.8 V) immediate froths
immediately from the bubbles
on all sets of electrodes → not
just DEP set.
conductive electrodes mostly.

bubbles due to conductive electrodes to
per pair on other lane w/ heater



= bubble on this set.
= bubble between DEP sets.

Small bubbles on electrodes.

Next section 100V

2.5V bonds. cc start capt

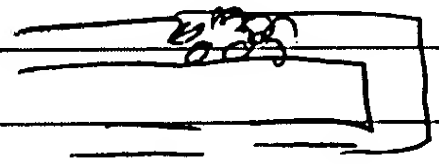
3.1 V stab.

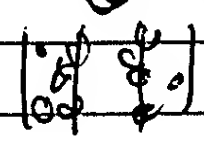
1.2V release

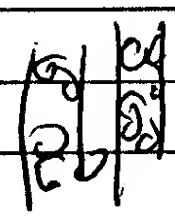
2.2V reception

small bubble rotated

conduit electrode bubble



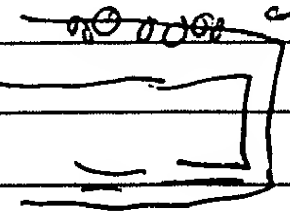




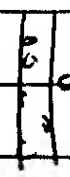
charred electrode at top of used DEP

at 100v, water for bubbles & no DEP

first start top of conductor seen 2-3μm



also fine coming in nearest cathode



Same dye w/ Bg, 100V 1 Khz > 10 Khz
up to 5 V

Bg 100V 3.8 V 10 Khz
/

= bare electrodes
100V 1 KHz 1 µs beads
8 V

no gnd connected
bubble still at inconsistent rate

→ Pt electrods 10/10

100 V

6 V 1 kHz
cell 1st sec when capture

200 V
→ boiling

bubbles → Hp electrode, Hp of 1st DEP, ~ pearl o/b
conduit on side
not by heater

Figure 8.1 Fluorescence emission sp
DNA-bound cyanine dimers

ATTACHMENT C

electro-osmotic flow

mixed 10 ml .1M NaCl with 1 drop 1um beads

When device hooked to high voltage resistance is ~200 KΩ (with solution in place)

Injected Solution/beads in one hole. Succeed all but 2 others (which were filled with solution/beads. After infusing/withdrawing several times got resistance down to 50kΩ but then kept climbing. It turned out, solution was leaking into other set of flow channels. Sealed them off.

At start of trip, resistance measured ~.6 MegΩ

EO Anodically bonded port Ti/Pt, no nitride resistance measures 800K-Ω
.1M NaCl with 1u beads - same conc as above
Applied 500 V saw minimal motion then pwr supply tripped off. Not all beads moving only 1 layer
May be getting electrolysis/hydrolysis
lets test

Small beaker of .1M NaCl put electrodes in solution - measured resistance

It's very erratic with alow [illegible]
.3 Megohm. Every time electrod
are removed then put back in
Solution the Value Changes
Substantially .3 Megohm [illegible]

Applied 500 V to beaker with
.1M NaCl in it. Within 1-2 sec
foaming & gas coming off one elec
small bubbles formed at the [illegible]
electrode. Beaker had ~ 1[4] [illegible]
Solution in it

lets try lower voltage - 250 V
- Same results

Lets try a more dilute NaCl
Solution 10 ml H_2O 2 ml .1MN
- Same results but after a few
seconds pwr supply tripped off
(pwr supply trips at 400 mA

Testing Cr/Pt heater ~~(could be [illegible] with ~~[illegible]~~ to took in cleanroom [illegible]~~

There should be ~ 2000Å Pt
Resistance of device ~ 330 Ω

~~| Voltage	temp
10V	29°C
15V	36°C
20V	40-44°C
24V ~~[illegible]~~ ~~25V~~	70-72°C
32V	|~~

above data not reproducible